IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Miri Seiberg, et al.

Serial No. **09/698,454**

Art Unit: **1616**

Examiner: **M. Lamm**

Attorney Docket No.: **JBP 518**

SOY DEPIGMENTING AND SKIN CARE COMPOSITIONS

DECLARATION OF MIRI SEIBERG, PH.D.

I, Miri Seiberg, am a Principal Research Fellow in the Skin Biology Technical Resource Center at Johnson & Johnson Consumer Companies, Inc. My education includes a Ph.D. in Molecular Biology from The Weizmann Institute of Science, Rehovot, Israel, in collaboration with Princeton University, Princeton, NJ and a B. S. in Life Sciences from Tel-Aviv University, Tel-Aviv, Israel. My curriculum vitae is attached hereto as Exhibit 1.

1. This Declaration is respectfully submitted to discuss how processing steps performed upon a legume extract affect the activity of proteins contained in the legume extract. Proteins are defined by both (1) their chemical structure, which includes its substituent amino acids as well as their unique conformation and (2) their biological function. A protein's biological function or activity requires the presence of both its chemical structure and conformation.

2. Proteins are said to be "denatured" when their physical and physiological properties are changed such that they lose their activity. Such change is generally due to a change in a protein's chemical structure and/or conformation. Protein denaturation and the consequent loss of biological activity are described in biochemistry textbooks (e.g. Biochemistry, A. L. Lehninger, 1975, p.62-63 (Exhibit 2).

3. Those knowledgeable about protein activity at the time the invention was made were aware that proteins are denatured in the presence of ethanol. A study of the interactions between the protein lysosyme and ethanol suggests a model for denaturation

of lysozyme by alcohol (Lehmann MS, Mason SA , McIntyre GJ .Study of ethanol-lysozyme interactions using neutron diffraction. Biochemistry. 1985 Oct 8;24(21):5862-9). A study on the effect of ethanol on proteins of the opioid receptor family showed that binding of the opioid ligands to the receptors was inhibited by ethanol because ethanol affected the conformation of peptides and caused denaturation of the receptor proteins, (Bhargava HN, Rapaka RS , Renugopalakrishnan V . Effect of ethanol on the binding of conformationally rigid and labile ligands of opioid receptors to rat brain membranes. Biochem Pharmacol. 1988 Jun 1;37(11):2279-83). Studying activity of proteins of the sodium channel family, it was demonstrated that ethanol and other alcohols cause an irreversible effect, eliminating the measured activity, because of the denaturation of the sodium channels. (Kukita F , Mitaku S Kinetic analysis of the denaturation process by alcohols of sodium channels in squid giant axon. J Physiol. 1993 Apr;463:523-43).

4. Soybeans are of high nutritional value, however they have been known to possess certain undesirable qualities which limit their use in animal and human nutrition. (e.g. Bau HM , Alais C Denaturation and enzymatic proteolysis in vitro of protein fractions of soya flour Ann Nutr Aliment. 1975;29(4):351-70). Numerous studies have evaluated the effects of technological treatments on the properties of certain soybean protein fractions, in order to identify a fraction with nutritional value but no gastro-intestinal side effects. The properties of such fractions were studied both before denaturation and after denaturation by either heat or alcohol, demonstrating the use of ethanol as a known agent that causes soy protein denaturation. (Bau HM , Alais C Denaturation and enzymatic proteolysis in vitro of protein fractions of soya flour Ann Nutr Aliment. 1975;29(4):351-70)

5. I have reviewed the Kelly reference set forth in the Office Action of June 16, 2005. In Example 1, Kelly sets forth a method of extracting a fraction of red clover to obtain isoflavones. This method of extraction requires the use of ethanol. Based upon my knowledge and understanding, and as documented in the references listed above, one of ordinary skill in the art employing such an extraction method would denature proteins contained in the plant. If applied to soybeans, this extraction method would denature proteins having serine trypsin protease activity and would, therefore, not produce a composition such as that set forth in the claims of the above-captioned application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Miri Seiberg

12/14/05

Date

Exhibit 1

Miri Selberg
168 Herrontown Rd. Princeton, NJ 08540
(908) 874-2325 (W); (609) 497-0148 (H)

Education

1977 B.Sc. Biological Sciences, Tel-Aviv University, Israel.
1982 M.Sc. Biochemistry, The Weizmann Institute of Science, Israel.
1989 Ph.D. Molecular Biology, The Weizmann Institute of Science, Israel, in collaboration with Princeton University, Princeton NJ.

Employment

1982 **The Weizmann Institute of Science, Israel.**
Research assistant, Dept. of Chemical Immunology.

1982-90 **Princeton University, Princeton NJ**
1982-84 Visitor, Dept. of Biochemical Sciences.
1987-89 Visitor, Dept. of Molecular Biology.
1989-90 Post Doctoral Fellow, Dept. of Biology.

1990- 92 **Bristol-Myers Squibb PRI, Princeton NJ.**
Post Doctoral Fellow, Dept. of Macromolecular Structure.

1992- **Johnson & Johnson**
1992-95 Senior Scientist, Skin Biology Research Center of PRI, Raritan NJ.
1995-96 Staff Scientist, Dermatology R&D, CPWW Skillman NJ.
1997-99 Principal Scientist, Skin Research Center, CPWW, Skillman NJ
1999-0 Research fellow, Skin Research Center, CPWW, Skillman NJ
2001 -05 Sr. Research fellow, Skin Biology TRC and LAS, CPWW, Skillman NJ
5/2005- Principal Research fellow, Skin Biology TRC and LAS, CPWW, Skillman NJ

Industrial Experience

1990- 92, Bristol-Myers Squibb PRI, Princeton NJ.

Post Doctoral Fellow, Dept. of Macromolecular Structure.

Using a rat model system for salt-induced hypertension, identified a novel gene involved in salt-induced hypertension, and demonstrated selective expression patterns.

1992-today, Johnson & Johnson

1992-95, Senior Scientist

This position involves conducting individual projects, supervising one BS/MS technician. Identified pathways involved in epidermal differentiation, hair growth and keratinocyte apoptosis. Developed relevant bioassays and screens.

1995-96, Staff Scientist

Directed two research scientists. Developed enzymatic, molecular and cellular assays and screens for potential drug and cosmetic activity. Involved in retinoid studies, proteases and protease inhibitors, in epidermal differentiation and hair growth.

1997-99, Principal Scientist

Head of pigmentation group. Directed research scientists and postdoctoral fellows. Horizontally directed the pigmentation technology development team. Initiated and directed molecular, cellular, and biochemical studies of pigmentation, resulting in the identification of a novel pathway that regulates skin color. Identified agents, both drugs and cosmetics, to modulate this pathway, resulting in darkening or lightening of human skin. Designed and evaluating product prototypes for biological activity and efficacy. In charge of numerous academic collaborations.

1999-00, Research Fellow

Continue heading the pigmentation team and supporting technology and product design groups in creating a line of depigmenting agents. First products available in stores. Additional responsibility in heading the hair growth efforts, introducing a new concept for delaying hair growth. Identified novel cosmetic agents with modulatory effect, demonstrated preclinical POP and initiated product development efforts. Expand responsibility for academic collaborations.

2001 -2005, Sr. Research Fellow

Director of the Skin Biology research group, including pigmentation, hair, acne, skin aging and skin cancer teams and supporting facilities. Continue basic research and product development support in all areas. Identified a novel cosmetic for skin aging, currently under early development stages. Directed efforts in the development of a new drug for acne, based on a proprietary target, now under clinical evaluation. Continue R&D support for skin lightening technology, now sold by numerous Brands and J&J companies worldwide. Continue R&D support for delaying hair growth technology, now sold by numerous J&J companies and Brands worldwide. Received the Johnson Medal, the highest level of scientific recognition by JJ. Head of Laboratory Animal Services, incl. vivarium support for numerous J&J companies. Council member of the J&J Corporate office of Science and Technology. In charge of academic interactions and collaborations for Skin Biology and related areas. In charge of the J&J SRC training grant. Member of the mentoring team.

5/2005, Principal Research Fellow

M. Seiberg

Patent applications

More than 25 patent applications in the areas of skin and hair

J&J Awards

1. Skin care council – best scientific content poster award. June 1993.
2. American Express achievement award of PRI. January 1995.
3. COSAT-CORD internship award. April 1997.
4. Skin care council – best overall poster award. June 1999.
5. COSAT excellence in science award. November 1999.
6. CPWW achievement award. January 2000.
7. Skin care council – best overall poster award. June 2001.
8. CPPW Grandview award. March 2003.
9. The Johnson Medal. Oct 2003.
10. The Mountainview award. March 2005.

Societies

1. Pan American Society of Pigment Cell Research (council member, 2001-03, member of finance committee, 2000-02, nominated for 2005 presidency elections).
2. Society of Investigative Dermatology
3. American Society of Cellular Biology
4. American Association for the Advancement of science
5. New York Academy of Science (elected 2003).

M. Seiberg

Publications

D. Duksin, **M. Seiberg** and W.C.Mahoney. (1982) Eur. J. Biochem 129: 77-80. Inhibition of protein glycosylation and selective cytotoxicity towards virally transformed fibroblasts caused by B3-Tunicamycin.

M. Seiberg and D. Duksin. (1983) Can. Res. 43: 845-850. Selective cytotoxicity of purified homologues of Tunicamycin on transformed Balb/3T3 fibroblasts.

S.F. Yu, T. Von Ruden, P.W. kantoff, C. Garber, **M. Seiberg**, U. Ruther, W.F. Anderson, E.F. Wagner and E. Gilboa. (1986) Proc. Natl. Acad. Sci. USA. 83: 3194-3198. Self inactivating retroviral vector designed for transfer of whole genes into mammalian cells.

P.W. Kantoff, D.B. Kohn, H. Mitsuya, D. Armertano, **M. Seiberg**, J. A. Zweibel, M.A. Eglitis, J.R.McLachlin, D.A. Wiginton, J.J. Hutton, S.D. Horowitz, E. Gilboa, R.M. Blaese and W.F. Anderson. (1986) Proc. Natl. Acad. Sci. USA. 83: 8563-8567. Correction of adenosine deaminase deficiency in cultured human T and B cells by retrovirus-mediated gene transfer.

M. Seiberg, M. Kessler, A.J. Levine and Y. Aloni. (1987) Virus Genes 1:1 97-116. Human RNA polymerase II can prematurely terminate transcription of Adenovirus type 2 late transcription unit at a precise site that resembles a prokaryotic termination signal.

M. Seiberg, Y. Aloni and A.J. Levine. (1989) J. Virol 63:1134-1141. The Adenovirus type 2 DNA binding protein interacts with the major late promoter Attenuator RNA.

M. Seiberg, Y. Aloni and A.J. Levine. (1989) J. Virol 63: 4093-4096. A comparison of human and monkey cells for their ability to attenuate transcripts that begin at the adenovirus major late promoter.

H. Cho, **M. Seiberg**, I. Georgieff, A.K. Teresky and A.J. Levine. (1989) Journal of Neuroscience Research 24: 115-122. The impact of the Genetic background of transgenic mice upon the formation and timing of chorold plexus papillomas.

M. Moore, A.K. Teresky, A.J. Levine and **M. Seiberg**. (1992) J. Virol 66(2): 641-649. P53 Mutations Are Not Selected for in Simian Virus 40 T-antigen-Induced Tumors from Transgenic Mice.

K.S. Stenn, L. Lawrence, D. Veis, S. Korsmeyer and **M. Seiberg**. (1994) Journal of Investigative Dermatology, 103: 107-111. Expression of the Bcl-2 Protooncogene in the Cycling Adult Mouse Hair Follicle.

M. Seiberg, J. Marthinuss and K. S. Stenn. (1995) Journal of Investigative Dermatology, 104(1): 78-82. Changes in Expression of Apoptosis - Associated Genes Mark Early Catagen.

J. Marthinuss, L. Lawrence and **M. Seiberg**. (1995) Cell Growth and Differentiation, 6: 239-250. Apoptosis in Pam212 Epidermal Keratinocytes: the Role of bcl-2 in Epidermal Differentiation.

M. Seiberg and J. Marthinuss. (1995) Developmental Dynamics, 202: 294-301. Clusterin Expression Within Skin correlates with hair growth.

J. Marthinuss, P. Andrade-Gordon and **M. Seiberg**. (1995) Cell Growth and Differentiation, 6: 807-816. A secreted serine protease can induce apoptosis in Pam212 keratinocytes.

R.J. Santulli, C.K. Derian, A. Darrow, K.A. Tomko, A. Eckhardt, **M. Selberg**, B. Scarborough and P. Andrade-Gordon. (1995) Proc. Natl. Acad. Sci., 92: 9151-9155. Evidence for the presence of a protease activated receptor distinct from the thrombin receptor in human keratinocytes.

M. Selberg, S. Wisniewski, G. Cauwenbergh and S. S. Shapiro. (1997) Dev. Dynamics, 208: 553-564. Trypsin-induced follicular papilla apoptosis results in delayed hair growth and pigmentation.

M. Selberg, P. Siock, S. Wisniewski, G. Cauwenbergh and S. S. Shapiro (1997) J. Invest. Dermatol., 109: 370-376. The effect of trypsin on apoptosis, utriculi size and elasticity in the rhino mouse.

L. P. Bernhofer, **M. Selberg** and K. M. Martin (1999). Toxicology In Vitro, 13: 219-229. The influence of the response of skin equivalents systems to topically applied consumer products by epithelial-mesenchimal interactions.

M. Selberg, C. Paine, E. Sharlow, M. Costanzo, P. Andrade-Gordon, M. Eisinger and S. S. Shapiro (2000). Exp. Cell. Res. 254(1): 25-32. The Protease-Activated Receptor-2 regulates pigmentation via keratinocyte-melanocyte interactions.

M. Selberg, C. Paine, E. Sharlow, M. Costanzo, P. Andrade-Gordon, M. Eisinger and S. S. Shapiro (2000). J. Invest. Dermatol. 115(2): 162-7. Inhibition of melanosome transfer results in skin lightening.

E. Sharlow, C. Paine, M. Eisinger S. Shapiro and **M. Selberg** (2000). J. Cell Sci. 113(pt 17): 3093-3101. The Protease-Activated Receptor-2 upregulates keratinocyte phagocytosis.

C. Paine, E. Sharlow, F. Liebel, M. Eisinger, S. Shapiro and **M. Selberg** (2001). J. Invest. Dermatol. 116(4): 587-595. An alternative approach to depigmentation by Soybean extracts *via* inhibition of the PAR-2 pathway.

M. Selberg, J-C Liu, L.. Babiarz, E. Sharlow and S. Shapiro (2001). Soymilk reduces hair growth and hair follicle dimensions. Exp. Dermatol. 10: 405-13.

G. Scott, AC Deng, C. Rodriguez-Burford, **M Selberg**, RJ Han, L. Babiarz, W. Grizzle, W. Bell, A. Pentland, (2001). Protease-activated receptor-2, a receptor involved in melanosome transfer, is upregulated in human skin by UV irradiation. . J. Invest. Dermatol. 117(6): 1412-20.

B.Z. Lin, L. Babiarz, F. Liebel, E. Roydon Price, D. Fisher, G. Gendimenico, and **M. Selberg** (2002). Modulation of Microphthalmia-associated Transcription Factor Gene Expression Alters Skin Pigmentation. J. Invest. Dermatol. 119(6): 1330-1340.

G. Scott, S. Leopardi, L. Parker, L. Babiarz, **M. Selberg**, R. Han (2003). The PAR-2 receptor mediates phagocytosis in a Rho-dependent manner in human keratinocytes. J. Invest. Dermatol. 121:529-541.

L. Babiarz-Magee, N. Chen, **M. Selberg** and B.Z. Lin (2004). The Expression and Activation of PAR-2 Correlate with Skin Color. Pigment Cell Res. 17: 241-251.

G. Scott, S. Leopardi, S. Printup, N. Malhi, **M. Selberg** and R. LaPoint (2004). Proteinase activated receptor-2 stimulates prostaglandin production in keratinocytes: analysis of prostaglandin receptors on human melanocytes and effects of PGE2 and PGF2α on melanocyte dendricity. JID, 122: 5, 1214-24.

M. T. Huang, J. G. Xie, C. B. Lin, M. Kizoulis, **M. Seiberg**, S. Shapiro, and A. H. Conney (2004) Inhibitory Effect of Topical Applications of Non-denatured Soymilk on the Formation and Growth of UVB-induced Skin Tumors. Oncology Res. 14(7-8):387-397.

Chapters, Invited Reviews

O. Resnekov, E. Ben-Asher, E. Bengal, M. Choder, N. Hay, M. Kessler, N. Ragimov, **M. Seiberg**, H. Skolnik-David and Y. Aloni. (1988) Gene 72: 91-104. Transcription termination in animal viruses and cells.

R. Quartin, M. Moore, **M. Seiberg**, C. Finlay, S. Chu, J. Martinez, D. Dittmer, J. Momand and A.J. Levine. Pezcoller Symposium, Trenton, Italy, (June 1991). The p53 Gene and Protein and Its Interactions with Viral Oncogene Products.

S. Prouty, **M. Seiberg** and K.S. Stenn. (1994) Journal of Dermatological Science 7 (suppl): 109-124. Molecules of Hair Follicle Cycling – A Tabulated Review.

M. Seiberg. (1997) Serine Proteases, apoptosis and PCD in skin and hair. In: Apoptosis: Practical applications and novel therapies, Chapter 3.3.1, IBC Press, USA.

M. Seiberg and S. Shapiro (1998) The regulation of pigmentation by serine proteases and their inhibitors. In: Inhibition of human proteases: from target identification to therapy, CHI Press, USA.

M. Seiberg . (2001) Pigment Cell Res 2000; 14: 236-242. Melanocyte and Keratinocyte Interactions in Melanosome Transfer.

C. Guttman, J.C. Liu, **M. Seiberg** (2001) Dermatology Times 22: 24.

M. Seiberg, (2002). in: JP Ortonne, R. Balotti (eds), Mechanisms of Tanning. Martin Dunitz. PAR-2 regulates pigmentation via melanosome phagocytosis. p. 215-228.

C. Paine, L. Bablarz, E. Sharlow, F. Liebel, M. Eisinger, S. Shapiro and **M. Seiberg**. (2001) An alternative approach to depigmentation by Soybean extracts *via* inhibition of the PAR-2 pathway. Proceedings of the 10th European Academy of Dermatology and Venerology.

M. Seiberg, L. Babiarz, J.C. Liu, and S.S. Shapiro. Soymilk Reduces Hair Growth and Follicle Dimensions. Proceedings of the 9th European Hair Research Society meeting (Invited, in press).

M. Seiberg, J.C. Liu, L. Bablarz, S.S. Shapiro, S. Ball, I-T Wu, Y. Appa. (2003). Soy extracts reduce hair growth and hair follicle dimensions. in: D. Van Neste (ed), Hair Science and Technology.

M. Seiberg. Skin pigmentation, lightening and darkening. Chapter 4. In R. Lad (ed), Biotechnology in Skin Care (04, in press).

J-C Liu, J Wu and **M Seiberg**. Applications of total soy in skin care. Chapter 12, p.115-127. In: Baran and Maibach (eds), Textbook of Cosmetic Dermatology (2004, in press).

M. Seiberg

Abstracts, conference presentations

M. Seiberg, A.J. Levine and Y. Aloni. CSH DNA Tumor virus meeting. (1985), p. 82. Attenuation in Ad2 as a mechanism of transcription-termination by RNA polymerase II.

Y. Aloni, O. Resnekov, **M. Seiberg**, M. Kessler and A.J. Levine. CSH RNA processing meeting (1986), p. 23. Eucaryotic RNA polymerase II can prematurely terminate transcription at precise sites that resemble a procaryotic termination signal.

M. Seiberg and Y. Aloni. CSH DNA Tumor virus meeting (1986), p. 128. Attenuation in Ad2 may determine its host-range.

Y. Aloni, M. Kessler, O. Resnekov, **M. Seiberg**, N. Ragimov, E. Bengal and O. Amster-Choder. CSH RNA processing meeting (1987), p. 21. Attenuation in the regulation of gene expression in virus and animal cells.

M. Seiberg, A.J. Levine and Y. Aloni. CSH DNA tumor virus meeting (1988), p.198. The 72kDa Ad2 DNA binding protein (DBP) specifically binds to the Ad2 Attenuated RNA.

M. Moore, A. Teresky, A.J. Levine and **M. Seiberg**. The Fifth Annual p53 Workshop, Princeton University (1991). The Characterization of p53 Expression in T-Antigen Induced Liver Tumors in Transgenic Mice: p53 Mutation is not Selected for During Tumor Development.

K.S. Stenn, L. Lawrence, D. Veis, S.J. Korsmeyer and **M. Seiberg**. 54th annual meeting of the Society for Investigative Dermatology, (1993). J. Invest. Dermatol. 100 (4) p. 512. Proto-oncogene bcl-2 RNA Expression in the Cycling Hair Follicle Correlates with Anagen.

J. Marthinuss, K.S. Stenn and **M. Seiberg**. 31st Annual meeting of the American Society of Dermatopathology, J. of Cutaneous Pathology 20(6), p. 557 (1993). P53 Appears Inessential to Hair Growth and Cycling in the Adult Mouse.

M. Seiberg, L. Lawrence, J. Marthinuss, D. Veis, S. Korsmeyer and K.S. Stenn. 1st International Conference on Applications of Apoptosis, Programmed Cell Death, Jan. 17-19, 1994, #40. Bcl-2 Expression in the Epidermis and the Cycling Hair Follicle (Invited speaker).

M. Seiberg, J. Marthinuss and K.S. Stenn. 55th Annual meeting for the Society of Investigative Dermatology, (1994). J. Invest. Dermatol. 102(4) p. 532. Pathways of Gene Expression Along the Hair Cycle (Invited speaker).

S.M. Prouty, **M. Seiberg**, J. Marthinuss, L. Lawrence and K.S. Stenn. 55th Annual meeting for the Society of Investigative Dermatology (1994). J. Invest. Dermatol. 102(4) p. 624. c-fos Expression Pattern Following Anagen Induction in the Plucked-Skin Mouse Model.

J. Marthinuss, K.S. Stenn and **M. Seiberg**. Molecular & Cell Biology of Apoptosis in Development, Disease Cancer (Sep. 1994), Apoptosis in Pam212 Epidermal Keratinocytes: the Role of bcl-2 in Epidermal Differentiation (Invited speaker).

M. Seiberg and J. Marthinuss. 56th Annual meeting for the Society of Investigative Dermatology. (1995). J. Invest. Dermatol. 104(4) p.638. Clusterin Expression Within Skin correlates with hair growth.

J. Marthinuss, P. Andrade-Gordon and **M. Seiberg**. (May 1995) A secreted serine protease can induce apoptosis in Pam212 keratinocytes. 2nd annual Conference on commercial prospects of Apoptosis.

M. Seiberg, S. Shapiro, S. Wisnievsky and G. Cauwenbergh (Nov 1996) Serine proteases, PCD and apoptosis in skin and hair. 3rd annual international conference on Apoptosis (Invited speaker).

M. Seiberg, S. Wisniewski, G. Cauwenbergh and S. S. Shapiro. (Apr 1997) Serine proteases, PCD and apoptosis in skin and hair. In: The biology of proteolysis, p. 127, CSHL Press.

M. Seiberg and S. Shapiro (Apr 1998). The regulation of pigmentation by proteases and their inhibitors. In: Inhibition of human proteases: from target identification to therapy (Invited speaker).

M. Seiberg and S. Shapiro (1998). Pigment Cell Research 11(3) p. 175. The regulation of pigmentation by serine proteases and their inhibitors. The 1998 PASPCR meeting (Invited speaker).

M. Seiberg and S. Shapiro. (Nov 1998). The effect of serine proteases and their inhibitors on pigmentation. 2nd international meeting of hair research societies (Invited speaker).

M. Seiberg (Feb 1999). The regulation of pigmentation by serine proteases and their inhibitors. 3rd international conference on cosmeceuticals (Invited speaker).

M. Seiberg, C. Paine, E. Sharlow, P. Andrade-Gordon, M. Costanzo, M. Eisinger and S. Shapiro (1999). Pigment Cell Research Supp 7, p. 41. The Protease-Activated Receptor-2 regulates pigmentation via keratinocyte-melanocyte interactions. IPCC International meeting of pigmentation (Invited speaker).

E. Sharlow, C. S. Paine, L. Babiarz, M. Eisinger, S. Shapiro and **M. Seiberg** (2000). J. Invest. Dermatol. 114 (4) p. 814. The Protease-Activated Receptor-2 upregulates keratinocyte phagocytosis.

B. Lin, C. Paine, F. Liebel, J. Mezick, G. Gendimenico and **M. Seiberg** (2000). J. Invest. Dermatol. 114 (4) p. 817. Using MITF to identify modulators of pigmentation.

M. Seiberg, E. Sharlow, C. Paine, L. Babiarz, M. Eisinger and S. Shapiro (2000). Pigment Cell Research 13 (5) p. 198. The Protease-Activated Receptor-2 affects pigmentation by upregulating keratinocyte phagocytosis (Invited speaker).

A. Deng, **M. Seiberg**, A. Pentland, R. Han and G. Scott (Oct 2000). The American Society of Dermatopathology 38th annual meeting. Protease-Activated Receptor-2 (PAR-2), a receptor involved in melanosome transport, is upregulated in skin in vivo by Ultraviolet irradiation.

E. R. Sharlow, C. S. Paine, L. Babiarz, M. Eisinger, S. Shapiro and M. **Seiberg** (Dec 2000). 40th Annual meeting of the American Society for Cell Biology. Molecular Biology of the Cell 11(suppl), p.228a. The protease-activated receptor regulates Keratinocyte Phagocytosis.

M. Seiberg, C. Paine, E. Sharlow, M. Eisinger and S. S. Shapiro (March 2001). 59th annual meeting of the American Academy of Dermatology. The PAR-2 pathway can regulate pigmentation.

C. Paine, E. Sharlow, F. Liebel, M. Eisinger, S. Shapiro and **M. Seiberg** (March 2001). 59th annual meeting of the American Academy of Dermatology. An alternative approach to depigmentation by Soybean extracts *via* inhibition of the PAR-2 pathway

M. Seiberg, C. Paine, J-C Liu and S. Shapiro (March 2001). 59th annual meeting of the American Academy of Dermatology. Soymilk and soybean-derived proteins delay hair growth and reduce hair size and hair pigmentation

C. Paine, R. Gallaghar, and **M. Seiberg** (March 2001). 59th annual meeting of the American Academy of Dermatology. Computerized image analysis for hair growth studies.

C. Paine, G. Payonk, and **M. Seiberg** (March 2001). 59th annual meeting of the American Academy of Dermatology. Computerized image analysis for pigmentation studies.

M. Seiberg, C. Paine, J-C Liu and S. Shapiro (March 2001). 59th annual meeting of the American Academy of Dermatology. Soymilk prevents UV-induced pigmentation and reduces sunburns.

J-C Liu, **M. Seiberg**, S. Shapiro and R. Grossman (March 2001). 59th annual meeting of the American Academy of Dermatology. Soy: Potential applications in skin care.

C Paine, L. Babiarz, E. Sharlow, F. Liebel, M. Eisinger, SS Shapiro and **M. Seiberg**., An Alternative Approach to Depigmentation by Soybean Extracts via Inhibition of the PAR-2 pathway. Society for Investigative Dermatology Annual Meeting (2001) abstract # 731 p.52.

M. Seiberg, L. Babiarz, J.C. Liu, and S.S. Shapiro. Soymilk Reduces Hair Growth and Follicle Dimensions. Society for Investigative Dermatology Annual Meeting (2001) abstract # 261 p.36.

C. Paine, E. Sharlow, L. Babiarz, F. Liebel, M. Eisinger, S. Shapiro and **M. Seiberg**. 10th congress of the European Academy of Dermatology and Venerology. An alternative approach to depigmentation by Soybean extracts *via* inhibition of the PAR-2 pathway (Oct 2001).

J.C. Liu, **M. Seiberg**, J. Miller and J. Wu. 10th congress of the European Academy of Dermatology and Venerology. Application of Soy in skin care (Oct 2001).

M. Seiberg, C. Paine, M. Eisinger and S. Shapiro. 10th congress of the European Academy of Dermatology and Venerology. The PAR-2 pathway can regulate pigmentation (Oct 2001).

J.C. Liu, **M. Seiberg**, J. Miller, J. Wu, S. Shapiro and R. Grossman. 4th International Symposium on the role of Soy in preventing and treating chronic disease. Applications of soy in skin care. (2001).

J.C. Liu, **M. Seiberg**, F. Liebel, T. Chen, Y. Appa, and T. Oddos. Pre-clinical and clinical evaluation of Total Soy preparations in improving skin physical tone parameters. 60th annual meeting of the American Academy of Dermatology. (Feb 2002).

C. B. Lin, L. Babiarz, F. Liebel, Roydon E. Price, D. E. Fisher, G. J. Gendimenico, and **M. Seiberg**. Modulation of microphthalmia-associated transcription factor expression alters skin pigmentation. Society for Investigative Dermatology Annual Meeting (Apr. 2002). J. Invest. Dermatol 119, p.339

L. Babiarz, C. B. Lin and **M. Seiberg**. Differential expression of PAR-2 and its activator trypsin in different skin types. Society for Investigative Dermatology Annual Meeting (Apr. 2002). J. Invest Dermatol. 119, p.340.

M. Seiberg, L. Babiarz, J.C. Liu, and S.S. Shapiro. Soymilk Reduces Hair Growth and Follicle Dimensions. European Hair Research Society 9th meeting (June 2002).

C. B. Lin, L. Babiarz, F. Liebel, Roydon E. Price, D. E. Fisher, G. J. Gendimenico, and **M. Seiberg**. Modulation of microphthalmia-associated transcription factor expression alters skin pigmentation. International Pigment Cell Conference (Sept. 2002).

L. Babiarz, C. B. Lin and **M. Seiberg**. Differential expression of PAR-2 and its activator trypsin in different skin types. International Pigment Cell Conference (Sept. 2002).

M. Seiberg, M. Filippi, A. Ricklis, V. Iotsova, C. B. Lin. Zinc Pyrithione Delays Hair Growth and Follicular Melanogenesis. International Investigative Dermatology Annual Meeting (Apr. 2003, #784).

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BIOCHEMISTRY

SECOND EDITION

THE MOLECULAR BASIS

OF CELL STRUCTURE AND FUNCTION

ALBERT L. LEHNINGER

THE JOHNS HOPKINS UNIVERSITY

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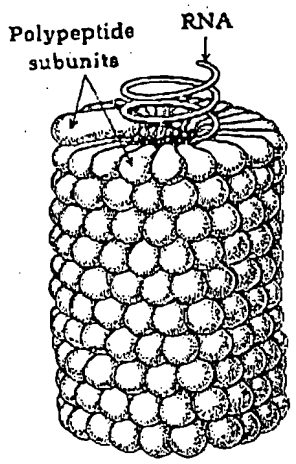
anywhere from two to twelve subunit chains in the smaller oligomeric proteins.

Since oligomeric proteins contain two or more polypeptide chains, usually not covalently attached to each other, it may appear improper or at least ambiguous to refer to such proteins as "molecules" and to speak of their "molecular weight." However, in most oligomeric proteins the separate chains are so tightly associated that the complete particle behaves in solution like a single molecule. Moreover, all the component subunits of oligomeric proteins are necessary for their biological function.

Supramolecular Assemblies of Proteins

Sometimes a set of protein molecules functioning together occurs in cells as a cluster or complex that can be isolated in homogeneous or even crystalline form. An example of a cluster of functionally related macromolecules, called a *supramolecular assembly* or *complex*, is the *fatty acid synthetase complex*, which contains one molecule of each of the seven different enzymes required for the biosynthesis of fatty acids (page 660). This complex can be isolated from yeast cells in homogeneous form (Table 3-2). The largest supramolecular protein complexes are the *viruses*, complexes of proteins and nucleic acids; some viruses also contain lipids and metal ions. *Tobacco mosaic virus* (Figure 3-4), one of the smaller viruses, has a particle weight of nearly 40 million, of which about 5 percent, or 2 million, consists of ribonucleic acid. The remaining 38 million is contributed by the protein portion, consisting of some 2,200 identical polypeptide chains. However, virus particles behave like single homogeneous structures having a definite molecular weight because their subunit components stick together very tightly.

Figure 3-4
Portion of a tobacco mosaic virus particle, a supramolecular assembly containing 2,200 polypeptide chains and a molecule of RNA.

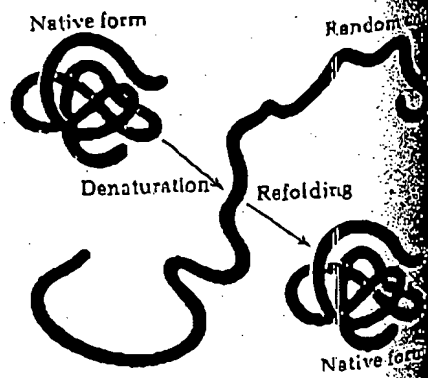


Denaturation

Most protein molecules retain their biological activity only within a very limited range of temperature and pH. Exposing soluble or globular proteins to extremes of pH or to high temperatures for only short periods causes most of them to undergo a physical change known as *denaturation*, in which the most visible effect is a decrease in solubility. Since no covalent bonds in the backbone of the polypeptide chain are broken during this relatively mild treatment, the primary structure remains intact. Most globular proteins undergo denaturation when heated above 60 to 70°C. Formation of an insoluble white coagulum when egg white is boiled is a common example of protein denaturation. But the most significant consequence of denaturation is that the protein usually loses its characteristic biological activity; e.g., heating usually destroys the catalytic ability of enzymes.

Denaturation is the unfolding of the characteristic native folded structure of the polypeptide chain of globular protein molecules (Figure 3-5). When thermal agitation causes the native folded structure to uncoil or unwind into a randomly looped chain, the protein loses its biological ac-

Figure 3-5
Denaturation and renaturation of a globular protein. After the polypeptide chain has been unfolded (by heating, by exposure to low pH, or by treatment with urea), it will often spontaneously refold to the native form.



tivity. Although each type of protein has an amino acid composition and sequence fixed during biosynthesis, the amino acid sequence as such does not *directly* endow a protein with its biological function or activity. However, we shall see that the amino acid sequence *ultimately* determines the biological activity of a protein because it determines the native conformation, or folded state, of the protein molecule, through interactions of the amino acid side chains with each other, with the solvent, and with other solutes. This conclusion follows from the discovery that denaturation, or unfolding, of native proteins into randomly coiled, biologically inactive forms is not irreversible, as was once thought. Many cases have now been observed in which an unfolded protein molecule spontaneously returns to its native biologically active form in the test tube, a process called *renaturation* (Figure 3-5). If the denatured protein was an enzyme, its catalytic activity returns on renaturation, without change in the specificity of the reaction catalyzed. However, renaturation of a denatured protein cannot evoke any biological activity that was not present in the original protein. These facts therefore indicate that the sequence of amino acids in the polypeptide chain contains the information required to specify its native folded conformation and that this native conformation determines its biological activity (Chapter 6).

The Functional Diversity of Proteins

Proteins have many different biological functions. Table 3-3 gives some representative types of proteins, classified according to function. The enzymes represent the largest class. Nearly 2,000 different kinds of enzymes are known, each catalyzing a different kind of chemical reaction. Enzymes have extraordinary catalytic power, far beyond that of man-made catalysts. They are highly specific in their function. The enzyme hexokinase catalyzes transfer of a phosphate group from adenosine triphosphate (ATP) to glucose, the first step in glucose metabolism. Other enzymes dehydrogenate fuel molecules. Still others, e.g., cytochrome *c*, transfer electrons toward molecular oxygen during respiration or, like DNA polymerase and amino acid–activating enzymes, participate in the biosynthesis of cell components. Each type of enzyme molecule contains an *active site*, to which its specific substrate is bound during the catalytic cycle. Many enzymes contain a single polypeptide chain; others contain two or more. Some enzymes, called *regulatory* or *allosteric* enzymes, are further specialized to serve a regulatory function in addition to their catalytic activity. Virtually all enzymes are globular proteins, as defined above. How enzymes catalyze chemical reactions is a major concern of modern biochemistry.

Another major class of proteins has the function of storing amino acids as nutrients and as building blocks for the growing embryo, e.g., *ovalbumin* of egg white, *casein* of milk, and *gliadin* of wheat seeds.

Some proteins have a transport function; they are capable of binding and transporting specific types of molecules via the blood. *Serum albumin* binds free fatty acids tightly and